REMARKS

Election/Restriction

In response to the final requirement restricting the claims, the nonelected claims are hereby canceled without prejudice to refiling these as part of divisional applications.

Claim Objections

Claim 60 was objected to under 37 C.F.R. § 1.75(c) as being of improper dependent form for failing to further limit the subject matter of claim 59. Claim 60 has been amended and in its amended form no longer depends from claim 59. In view of the amendment to claim 60 it is requested that this objection be withdrawn.

Claim 47 was objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form. Applicants urge below that the base claim is patentable. No amendment has been made to claim 47.

Rejections Under 35 U.S.C. § 112, second paragraph

Claim 46 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite because of the phrases "strong promoter" and "weak promoter". The Examiner suggested changing

this language to "high affinity" and "low affinity". Claim 46 has been amended as suggested by the Examiner. In view of this amendment it is urged that claim 46 is no longer indefinite and it is requested that the rejection under 35 U.S.C. § 112, second paragraph, be withdrawn.

Rejections Under 35 U.S.C. § 112, first paragraph

Claim 42 was rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter not enabled by the disclosure. The rejection was made in view of the claim requiring that the transcription factor is a glucocorticoid receptor, the Office Action asserting that the use of the complete glucocorticoid receptor for the claimed method was not enabled. The claim has been amended to require only that the transcription factor comprise a hormone binding domain of a glucocorticoid receptor, thereby eliminating the requirement for use of the complete glucocorticoid receptor. The Office Action itself pointed to language at column 3, lines 62-65 of issued U.S. Patent 6,063,985, as teaching the use of the hormone binding domain as a regulatory domain in a chimeric transcription factor. It is therefore urged that claim 42 as amended is enabled.

Claim 45 was rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Claim 45 is drawn to a vector comprising mutant *lox* sites which have lower affinity for CRE than do wild-type *lox* sites. The Office Action states that the prior art Albert et al. reference teaches that mutant *lox* sites with reduced binding affinity for the recombinase favors the stabilization of an integration event into a plant genome over the reverse excision event. The Office Action next states that this would impede one from using the claimed invention to excise the genes flanked by the recombination sites. Applicants disagree with this conclusion.

First, exactly as stated in the Office action, the Albert et al. reference teaches mutant *lox* sites that favor integration over excision. This does not mean that excision does not occur. It indeed does occur even with the mutant *lox* sites taught by the Albert et al. reference. This is most easily seen in Table 1 on page 652 of the reference in which the forward and reverse reaction rates are compared. For each set of mutant *lox* shown in the table, the forward reaction is favored at low concentrations of CRE but the difference between the forward and reverse rates decreases as the amount of CRE is increased, at least for the first two sets of data shown in the table and partially so for

the third set of data. The data in Table 1 of the Albert et al. reference illustrate the idea behind claim 45, i.e., one uses a mutant *lox* so that at low recombinase levels the forward (integration) activity is favored and at higher levels the reverse reaction becomes more favored than it had been although it does not necessarily become more favored than the forward reaction, rather it just becomes more likely that the reverse reaction will occur. The application explains this on page 10, lines 9-12, wherein it is stated that the use of mutant *lox* sites makes it more likely that eviction of the transgene will not occur (i.e., integration or the forward reaction is favored) until after induction of CRE at which point the higher levels of CRE begin to increase the amount of the reverse reaction (eviction) and make it more likely that eviction occurs at the desired timepoint. It is urged that in view of the present disclosure and the teaching of the Albert et al. reference that one of skill in the art would know how to make and use the claimed invention.

Claim 46 was rejected under 35 U.S.C. § 112, first paragraph, as being enabled only for use under airtight conditions. Applicants point out that the claim is drawn to a composition of matter, not to a method. The making of the

composition was not objected to, rather the ability to use the composition was called into question by the Office Action. The Office Action objects to enablement of using the composition for treating plants. The Office Action in the first sentence of the rejection admits that use of the claimed composition is enabled when using the GVG system under airtight conditions. Although applicants urge that the disclosure enables even more than that, it is urged that this is an admission by the PTO that the disclosure has enabled at least one use of the claimed composition. It is urged that a disclosure need enable only a single use of a composition to meet the statutory requirements of utility and enablement of how to use and make a composition; it is not required that every possible use of a composition be enabled by a disclosure in order to claim the composition. Engel Industries, Inc. v. Lockformer Co., 20 U.S.P.Q.2d 1300, 1304 (Fed. Cir. 1991).

In view of the amendments to the claims and in view of the above arguments, it is requested that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

Rejections Under 35 U.S.C. § 103(a)

Claims 39-44, 59, 60 and 72 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Ebinuma et al., in view of Lyznik et al. and Aoyama et al. The Patent Office has characterized the claims as "broadly drawn" towards a vector, yet goes on to list no less than seven material limitations to the claim. Applicants first wish to state their belief that the Examiner's characterization of the claim as "broad" misrepresents the very specific nature of the invention, and the precision with which it is claimed, and unnecessarily complicates the analysis.

The Patent Office has deemed it prima facie obvious to modify the method of marker gene excision of Ebinuma by placing a recombinase gene under the control of an inducible promoter as suggested by Lyznik et al. Paper 9, page 11. The Examiner asserts as motivation for making this combination the statement in Lyznik that "the desired products of the recombination would not stabilize unless the recombinase is regulated, for example with an inducible promoter." Id. The Examiner further argues that it would have been prima facie obvious to modify the method using the GVG system of Aoyama et al., given its advantages as taught by that reference. Paper 9, page 12. The Examiner also asserts, without providing any supporting reasons, that it would have been obvious to place the transcription factor gene within

the segment that is removed, as it would be no longer required after the removal of the genes that regulate. Id.

Applicants respectfully submit that this rejection is unwarranted. "In determining the propriety of the Patent Office case for obviousness in the first instance, it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the reference before him to make the proposed substitution, combination or other modification." In re Lintner, 458 F.2d 1013, 1016, 173 USPQ 560,562 (C.C.P.A. 1972). The Federal Circuit recently emphasized the criticality of the motivation element in the obviousness analysis, stating

> "[o]ur caselaw makes clear that the best defense against hindsight-based obviousness analysis is the rigorous application of the requirement for a showing of a teaching or motivation to combine the prior art references."

Ecolochem, Inc. v. Southern California Edison Co., 56 U.S.P.Q.2d 1065, 1073 (Fed. Cir. 2000). In order to prevent the use of hindsight, the Federal Circuit

> "requires the examiner to show a motivation to combine the references that create the case of obviousness. In other words, the examiner must show reasons that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements

> from the cited prior art references for combination in the manner claimed."

In re Rouffet, 47 U.S.P.Q.2d 1453, 1457-58, accord Ecolochem, 56 U.S.P.Q.2d at 1076. The rigorous application of the motivation element "stands as a critical safeguard against hindsight analysis and rote application of the legal test for obviousness." In re Rouffet, 47 U.S.P.Q.2d 1453, 1458 (Fed. Cir. 1998). Applicants submit that the Patent Office has failed to establish the motivation element of the obviousness test.

Ebinuma, et al. teaches a very specific vector system, with very narrow applicability. In Ebinuma, an unregulated transposase/recombinase is used to excise a morphological marker (the marker causes abnormal growth in the plant expressing it). The transposase/recombinase itself is not affected. Ebinuma describes very specific advantages of this system, the primary one being safety, because the use of a morphological marker (as opposed to an antibiotic resistance or other chemical marker) does not present the risk of toxicity due to the continued expression of such a marker in the transgenic product. Col. 12, lines 8-14. Other advantages taught are the overcoming of the problem of low excision frequency, because the use of the morphological marker provides a very clear and simple means of distinguishing plants in which excision has occurred (by their

reversion to a normal phenotype), col. 11, line 64- col. 12, line 7, and the ability to positively identify excision/ transposition events by the evident reversion to normal phenotype (i.e., by selecting only transgenic products that first acquire the abnormal phenotype of the morphological marker, then lose it, selection of (a) transgenic plants (b) with the maker excised is assured). Col. 9, lines 41-47; col. 12, lines 32-38. None of these advantages has relevance in a system that does not use both a morphological marker and the unregulated transposase/recombinase system of Ebinuma. Ebinuma, et al. in fact expressly teaches away from the use of anything but the morphological marker disclosed therein. See col. 2, lines 54-58; col. 7, lines 33-37. Therefore, there is absolutely no motivation to modify the Ebinuma reference in any way, because the specific advantages of the system would be lost (with use of anything but the morphological marker), or no particular benefit or advantage would be gained (with use of a regulated recombinase, for instance).

The Lyznik, et al. reference is likewise of very narrow scope. The problem addressed by the Lyznik reference is the issue of the reversibility of the recombination reaction, especially when the result is an inversion or transposition

rather than an elimination of the gene sequence. See Lyznik page 178, column 1, second paragraph. The passage indicated by the Examiner, that "the desired products of recombination cannot be stabilized within the chromatin structure unless the activity of the recombinase system is regulated," specifically addresses the need for regulation with regard to insertion, inversion, or transposition. Mention of excision is conspicuously absent. Furthermore, the issue of stabilization does not even come into play when the sequence is being excised, because the excised sequence is no longer present, and therefore the reverse reaction, i.e., insertion of the fragment, would not occur. Thus, the alleged motivation in Lyznik to combine with Ebinuma does not, in fact, exist.

Furthermore, the system taught by Lyznik, et al. suffers from a variety of deficiencies that would preclude its use in the kind of system contemplated by the present invention, a further disincentive to combine the references in the manner suggested. First, the system is very "leaky," meaning that it is not tightly regulated, nor particularly reliable in remaining "silent" when needed, and active when desired. Thus, if one were to, for the sake of argument, desire to achieve some greater degree of control over a system such as that disclosed in Ebinuma, et al.

(though such control is unnecessary; col. 7, lines 48-65; col. 7, line 66 to col. 8, line 14), the inducible promoter system described in Lyznik would not be suitable. Furthermore, Lyznik, et al. themselves concede that the ability to "turn on and off at will" site-specific recombination systems had not been attained with their system:

> "Once site-specific recombination can be turned on and off at will, their applications will be extremely valuable for the genetic engineering of crop plants and for basic studies of cell biology."

Page 183, sentence bridging col. 1 and 2 (emphasis added).

This statement in Lyznik clearly shows that the authors themselves did not believe that they had yet achieved a high degree of control over this system. Because the teachings of Lyznik do not overcome the deficiencies of the Ebinuma system, nor address the problems solved by the present invention, and because the suggested combination would not provide any particular advantage or benefit, a person of ordinary skill in the art would have no motivation to combine the teachings of the two references. Rouffet, 47 U.S.P.Q.2d at 1458; Ecolochem, 56 U.S.P.Q.2d at 1076.

The Aoyama reference does not make up for the deficiencies in the Ebinuma and Lyznik references. There is nothing in the Aoyama reference to suggest the use or suitability of the GVG

inducible promoter system for controlling a recombinase in a marker excision system such as that of the present invention. The Aoyama reference thus adds nothing significant to the reaching of Ebinuma and Lyznik and does not provide the motivation to make the suggested combination.

Furthermore, there are significant secondary indicia of the nonobviousness of the present invention. Significant unexpected results are achieved with the present system that could not be predicted from the prior art. The present system allows for effective excision in germ-line cells, permitting transmission of the "excised" phenotype to subsequent generations, and assuring that subsequent generations will be free of both the marker and the recombinase. In the prior art, excision was only carried out in somatic cells, and there is no indication, nor reasonable expectation, that the system would be effective in causing excision in the deep L2 layers of cells from which germ cells arise. Also, a high degree of excision frequency can be obtained with the present system, while inefficiency, unreliability, and low excision rate are all emphasized in the prior art. A tight and reliable control of excision is obtained with the present system, in contrast to the very "leaky" and unreliable excision seen in, for example, the Lyznik reference. The present system

virtually eliminates regression, problems highlighted in both the Ebinuma and the Lyznik references. Because the system of the present invention permits the complete removal of all the components of the marker and recombinase systems, it virtually eliminates cytotoxicity connected with the use of a marker gene and of the recombinase, something which cannot be predicted or reasonably expected from the systems of the prior art. Indeed, the issue of cytotoxicity due to continued expression of the recombinase (particularly after the excision event) is not even considered in the cited references, or recognized as a problem.

There is also evidence of a long-felt but unmet need in the prior art. Both Lyznik and Ebinuma have effective dates of 1995, four years prior to the filing date of the present application. The issues of reliability, stability, transmissibility and efficiency are all raised in the prior art, yet not adequately met by the systems disclosed therein, nor any disclosure between 1995 and the priority date of the present application. Lyznik, in fact, expressly states that the goal of "turning on and off at will" a recombinase gene had yet to be obtained. And still, with these disclosures before them for over four years, no person skilled in the art prior to the present inventors was led to make the combinations and modifications suggested by the Examiner to

arrive at the present invention. All of these secondary indicia of nonobviousness further demonstrate that the present invention is not obvious over the prior art. Rouffet, 47 U.S.P.Q.2d at 1456.

CONCLUSION

In view of the forgoing amendments and remarks, Applicants submit that the claims are in condition for allowance, and requests withdrawal of all pending rejections and a favorable action on their application.

Respectfully submitted,

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Amended claims: Version with markings to show changes made

42 (amended). The vector of claim 39 wherein said transcription factor [is] comprises a hormone binding domain of a glucocorticoid receptor.

46 (amended). The vector of claim 39 wherein said marker gene is under the control of a [strong] high affinity promoter and said inducible gene is under the control of a [weak] low affinity promoter wherein said [strong] high affinity promoter is induced by an inducer at a low concentration and said [weak] low affinity promoter is induced by said inducer at a high concentration.

60 (amended). [The method of claim 59] A method for excising a marker gene from the genome of a transgenic plant or plant cell after selection of said transgenic plant or plant cell, comprising:

a) transfecting a plant or plant cell with the vector of claim 39 to form said transgenic plant or plant cell;

b) selecting said transgenic plant or plant cell; then

c) adding an inducer to induce said inducible gene,

wherein said inducible gene produces a recombinase which removes said marker gene from said genome [wherein said transgenic plant or plant cell is selected prior to adding inducer].